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14. ABSTRACT

In the short-term, bacteria isolated from the same marine biofilms as diatoms caused the diatoms to form cellular aggregates, to lose their motility and their ability to adhere to surfaces. Longer incubation times lead to diatom lysis. A result of these actions was the reduction in structural stability of the mixed species biofilm to hydraulic stress. The active compound(s) from the bacteria was found to be secreted into the bacterial growth medium (MW > 10K Daltons) There is sufficient evidence to regard its mode of action as being lectin-like. Experiments with plant-derived fluorescently-conjugated lectins showed that the extracellular polymers of several diatoms stained specifically indicating the presence of galactose, glucose and mannose moieties, but not those of fucose. Our results are significant in terms of the use of bioassays to test the efficacy of candidate antifouling surfaces and studies of littoral sediment stabililization.

15. SUBJECT TERMS
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Cell-Cell interactions, Extracellular Matrix Formation in Biofilms and Marine Fouling

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LONG-TERM GOALS

To add to our understanding of the individual and joint roles of bacteria and diatoms in the formation and persistence of marine fouling biofilms and in so doing, provide information that can be used in the design of fouling release coatings. This information concerning the physiological dissection of the biofilm system is generally relevant to other mixed species biofilms, especially those on illuminated marine sediments in the littoral zone.

SPECIFIC OBJECTIVES

To determine in a mixed diatom/bacterial biofilm, which group of organisms influences the production of the extracellular matrix (ECM) that is responsible for biofilm architecture and persistence.

APPROACH

Eukaryote-prokaryote interactions lead to changes in the detection of a surface and potentially none attachment and /or detachment of cells, thus affecting biofilm structure and function. Bacteria and diatoms isolated from the same marine biofilm were used to study this phenomenon. Diatom motility (an analogue of adhesion) and adhesive behavior was monitored as a function of the presence of bacteria and their products. Efforts to quantify the ECM production in single and mixed organism biofilms and to determine its origin were made. Sugar-specific lectins conjugated to fluorophores were used to demonstrate the presence or absence of particular extracellular polymers. Flow-through bioreactors were used to assess biofilm the formation of extracellular products as a function of time using axenic diatom and mixed bacterial/diatom cultures. We also prepared extracellular polymers from diatoms and diatom-colonized surfaces for study with atomic force microscopy by another grantee.

RESULTS AND ACCOMPLISHMENTS

Bioreactor studies showed that diatom biofilms were less

stable in the presence of bacteria than they were in their absence. Specifically Pseudoalteromonas sp.4 (16-S RNA measurements indicate a new species) alone, or the medium in which it was grown, caused diatoms to lose motility, adhesion and viability.

As an initial effect, the spent medium caused diatoms (*Amphora coffeaeformis* and *Navicula* sp. 1)to agglutinate (fig1). Extracellular polymers of these diatoms were easily stained with various conjugated lectins, especially those specific for galactose and mannose, but after treatment with bacteria spent medium, the extracellular polymers were not detectable with the lectin.

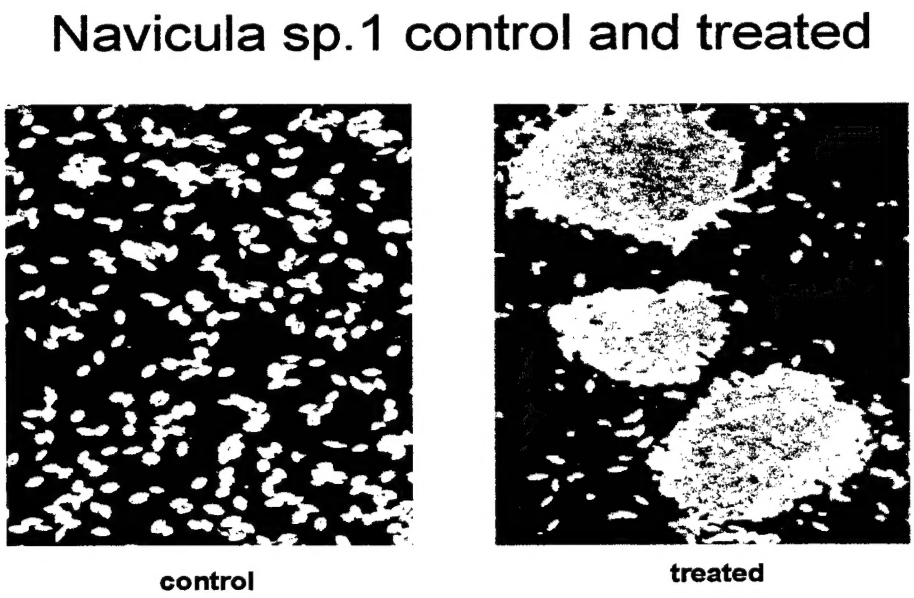

Figure 1. Agglutination effects of Pseudoalteromonas sp.4 medium on Navicula sp.1
Cells of Navicula sp. 1 were treated with spent medium (treated) from Pseudoalteromonas sp. 4. The left hand image (untreated) shows typical active dispersal behavior of diatom cells in fresh marine medium. In the right hand image the effect of their re-suspension in medium that had supported bacterial growth can be seen. The 'spent medium' was sterilized by ultra-filtration before the diatoms were re-suspended.

We found that sugars or their polymers(Table 1)protected diatom cells from the effects of the bacterial spent medium. This is strong evidence that the bacterial products capable of agglutinating the diatoms are lectins. A model to explain this phenomenon has been formulated.

Table 1. D-galactose or mannan protects *Navicula* sp.1 from effects of *Pseudoalteromonas* sp. 4

Treatment of diatoms	% motility (n) [1]
(a). ASP_2 C alone	98 ± 2 (101)
(b). Spent medium from bacteria	42 ± 12 (25) [2]
(c). as (b) + 50mM D-galactose	71 ± 7 (46)
(d). as (b) + 7 mg mL^{-1} mannan	66 ± 1 (38)

[1]After 120 min incubation, (number of cells measured).
Cell density was identical in each experiment, but aggregation of diatoms in the presence of the spent medium of *Pseudoalteromonas* sp. 4 reduced the number of individual motile cells able to be measured. Thus these figures represent the motility of cells not in an aggregate.

[2] After 180min motility in (b) was reduced to 0% whereas in the other treatments (a, c, and d), motility did not change.

We have shown that lectin staining of diatom extracellular polymers demonstrate differing adhesive structures between species. For instance, whereas *Amphora* [two raphes on the ventral valve] adheres to a surface by two raphe-penetrating pads of polymer, the adhesive pad for *Navicula* sp.1 covers the entire ventral valve surface interface. The adhesive material is obviously secreted from the raphe in *Amphora* but in *Navicula* sp.1 we postulated that it was secreted from valvar pores, i.e., not from the raphe. We have now documented that this is the case.

IMPACT/APPLICATIONS

The use of a single organism type in assessing antifouling coating formulations is not recommended since not all diatom species behave similarly and the influence of indigenous bacteria on the diatom adhesive behavior can be substantial.

HONORS

During the course of this funding period Keith Cooksey has been Chair Elect, Chair and Advisor to section Q of the American Society of Microbiology which deals with Environmental General and Applied Microbiology.

Keith Cooksey has been appointed to Emeritus Status at Montana State University-Bozeman- the first Research Professor at MSU to be so honored.

PUBLICATIONS AND PUBLISHED ABSTRACTS:

Articles

2001

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Abstracts

2001.

Oxygen tension in the benthic boundary layer : Water clarity, sediment stability and the activity of benthic phototrophs, in "Effects of Hypoxia on Aquatic Biota", [with B.Wigglesworth-Cooksey], January, La Paz, Mexico.

Diatom and bacterial extracellular polymers in sediment stabilization, [With B. Wigglesworth-Cooksey], Division of Biogeochemistry, American Chemical Society, April, San Diego, CA.

2002.

Diatom-bacterial interactions in marine biofilms and their significance in the design of fouling - release coatings,(with B. Wigglesworth-Cooksey). Annu.Mtg.Amer.Soc.Microbiol. Salt Lake City, April.

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Microphytobenthos, Amsterdam, August.

2004

Involvement of calcium-based signal transduction and fluxes in biofilm formation and cellular dispersal by benthic marine diatoms. (with B. Wigglesworth-Cooksey), Annu.Mtg.Amer.Soc.Microbiol New Orleans, May.